Appendix A

Species Listing PROPOSAL Form:

Listing Endangered, Threatened, and Special Concern Species in Massachusetts

Scientific name: _***Ambystoma laterale***_____ Current Listed Status (if any): _**Special Concern**___

Common name: _**Blue-spotted Salamander**_____

Proposed Action:

_____Add the species, with the status of: _____ Change the scientific name to: _____
_____Remove the species Change the common name to: _____
__**X**__Change the species' status to: (Please justify proposed name change.)
Threatened – Bristol & Plymouth counties
Special Concern – remainder of state

Proponent's Name and Address:
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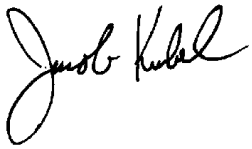
Proponent's Signature:  Date Submitted: **7/5/2018**

Please submit to: Natural Heritage & Endangered Species Program, Massachusetts Division of Fisheries & Wildlife, 1 Rabbit Hill Road, Westborough, MA 01581

Justification

Justify the proposed change in legal status of the species by addressing each of the criteria below, as listed in the Massachusetts Endangered Species Act (MGL c. 131A) and its implementing regulations (321 CMR 10.00), and provide literature citations or other documentation wherever possible. Expand onto additional pages as needed but make sure you address all of the questions below. The burden of proof is on the proponent for a listing, delisting, or status change.

(1) Taxonomic status. Is the species a valid taxonomic entity? Please cite scientific literature.

Yes. *Ambystoma laterale* was described by Hallowell (1856) and continues to be treated as a distinct taxonomic entity with no recognized subspecies (Petranka 1998, Highton et al. 2017). The species is a member of a salamander complex also containing *A. jeffersonianum*, *A. texanum*, *A. tigrinum*, *A. barbouri*, and an ancient, nearly all-female lineage of hybrid origin whose genetically diverse forms are known collectively as "unisexual *Ambystoma*" (Dawley and Bogart 1989, Petranka 1998, Bogart et al. 2009, Bi and Bogart 2010). The unisexual *Ambystoma* procreate via a complex reproductive system termed kleptogenesis, which relies on the "stealing" of sperm from sympatric males of the aforementioned sexual species, including *A. laterale* (Bogart et al. 2007, Bi et al. 2008). Unisexual associates of *A. laterale* were formerly treated as a

distinct species, *A. tremblayi* (Uzzell 1964), but that practice was generally discontinued (Lowcock et al. 1987, Petranka 1998). Dubois and Raffaëlli (2012) proposed assignment of all forms of unisexual *Ambystoma* to a single taxon (*A.* kl. *platineum*), but the unisexual *Ambystoma* are not currently recognized as distinct species or subspecies by Highton et al. (2017). Rather, unisexual *Ambystoma* are presently viewed as hybrid "forms" of the sexual species with which they interact in a given population, which seems justified given that unisexual reproduction sometimes does involve incorporation of genomes from males of the sexual species (Bogart and Klemens 1997; Bi and Bogart 2006, 2010; Bogart et al. 2007; Bi et al. 2008). Populations of *A. jeffersonianum* and *A. laterale* appear to be allopatric at the local scale in Massachusetts (Bogart and Klemens 1997, 2008; Charney et al. 2014; Kubel 2016; NHESP unpublished data), and so unisexual *Ambystoma* are treated taxonomically as a form of *A. laterale* wherever they occur in local populations of that species. That practice is further justified in that the *laterale* genomes found in individual unisexual *Ambystoma* salamanders are consistently derived from local or nearby populations of *A. laterale* (Bi et al. 2008).

(2) Recentness of records. How recently has the species been conclusively documented within Massachusetts?

Genetic sampling confirmed the presence of *A. laterale* at multiple sites among southwestern, central, and eastern Massachusetts during 2015–2017 (Kubel 2016, NHESP Database 2018). Additional observations of the species in Massachusetts were documented as recently as April 2018 (J.E. Kubel personal observation).

(3) Native species status. Is the species indigenous to Massachusetts?

Yes. *A. laterale* is native to Massachusetts and other states (CT, ME, NH, NJ, NY, VT) in the Northeast, as well as the Great Lakes region and Canada (Petranka 1998). Museum specimens from Massachusetts date at least as far back as the mid- to late 1800s (e.g., MCZ #972). The species is believed to have rapidly recolonized Massachusetts and other northern states less than 18,000 years ago from at least one refugium in eastern North America, following retreat of the Laurentide Ice Sheet during the last glacial maximum (Demastes et al. 2007, Bi et al. 2008).

(4) Habitat in Massachusetts. Is a population of the species supported by habitat within the state of Massachusetts?

Yes. Based on occurrence data (NHESP Database 2018) and other accounts (Klemens 1993, Downs 1989, Petranka 1998), *A. laterale* requires (a) terrestrial habitat in the form of woodlands associated with sandy glacial deposits, near or bordering large forested swamps, bogs, or floodplain marshes, and (b) aquatic breeding habitat in the form of ephemeral pools, shrub swamps, and other generally fishless wetlands. Such habitat configurations are common in eastern Massachusetts and some parts of the southern Connecticut River Valley and lower Housatonic River region, though most are fragmented by roads, commercial and residential development, agricultural fields, and other land alterations.

(5) Federal Endangered Species Act status. Is the species listed under the federal Endangered Species Act? If so, what is its federal status (Endangered or Threatened)?

No, the species is not listed under the federal Endangered Species Act.

(6) Rarity and geographic distribution.

(a) Does the species have a small number of occurrences (populations) and/or small size of populations in the state? Are there potentially undocumented occurrences in the state, and if so, is it possible to estimate the potential number of undocumented occurrences?

Yes. There are approximately 159 local populations of *A. laterale* currently considered extant (confirmed within the past 25 years) in Massachusetts, which excludes 6 populations that have very likely been extirpated and another 30 populations not observed/reconfirmed in 25–40 years (NHESP Database 2018; Figure 1). In contrast, the state supports thousands of local populations of Spotted Salamander (*A. maculatum*), the most closely-related species not considered rare or uncommon. Evidence of breeding by *A. maculatum* has been

documented at over 4,800 vernal pool basins in Massachusetts during the past 30 years (NHESP Database 2018), which represents only a fraction of the habitat available. Why *A. laterale* is so rare compared to *A. maculatum* is not fully understood, but relative habitat preferences vs. availabilities between the species may explain much of the difference.

Reliable data on population sizes of *A. laterale* in Massachusetts are scant, owing to the extraordinary expense and labor required to sample populations adequately. Minimum population sizes for some sites can be gleaned from research studies (e.g., Windmiller et al. 2008, Charney et al. 2014, Kubel 2016) that involved the marking of adult salamanders captured throughout a breeding period, and relative abundance may be inferred from counts of egg masses during routine surveys (NHESP unpublished data). Based on those works and anecdotal accounts, typical population sizes in Massachusetts appear to range from dozens to hundreds of individuals per site. However, some attempts to sample adult salamanders fail to detect more than several individuals (Charney et al. 2014, Kubel 2016, NHESP unpublished data), suggesting small population sizes. Small egg-mass counts also suggest small population sizes at some sites (NHESP unpublished data).

There are undoubtedly some undocumented local populations of *A. laterale* in Massachusetts. The species has low detection probability relative to other taxa (e.g., songbirds), especially outside its breeding season, which lasts just several weeks. Considerable inventory work (egg-mass and/or adult salamander surveys among hundreds of sites) has been completed by NHESP staff, research contractors, environmental consultants, volunteers, and others throughout the state during the past several decades (NHESP unpublished data). That work has provided a good understanding of the state distribution of *A. laterale*, but there are still patches of apparently suitable habitat that have not been surveyed adequately to conclude the species' absence. The NHESP has been using a preliminary species distribution model developed by Lori Johnson in 2013 to help identify sites of potentially undocumented populations, but the model was limited by taxonomic uncertainties associated with the species occurrence input data. Those uncertainties have since been resolved (Kubel 2016, NHESP unpublished data), and a future refinement of the model could better facilitate an estimate of the number of likely suitable but under-surveyed sites. In the meantime, I speculate that the number of undocumented populations of *A. laterale* in Massachusetts likely ranges in the dozens (as opposed to hundreds), based on the limited geographic distribution of the species, the amount of survey work to date, and the amount of apparently suitable habitat still available.

(b) What is the extent of the species' entire geographic range, and where within this range are Massachusetts populations (center or edge of range, or peripherally isolated)? Is the species a state or regional endemic?

The global range of *A. laterale* occurs in southern Canada and the northern United States, from Labrador south to New Jersey and west through New York, southern Ontario, northern Ohio, Indiana, and Illinois, to Minnesota and eastern Manitoba, with a population disjunction occurring in Iowa (Petranka 1998). Massachusetts is considered to be near, but not at, the southern edge of the range, as *A. laterale* appears to be absent from Rhode Island and much of eastern Connecticut and western Long Island.

The Massachusetts distribution of *A. laterale* appears to occur in five distinct regions: (1) *Southeast*, comprising northern Bristol and Plymouth counties; (2) *Northeast*, comprising Essex, Middlesex, Suffolk, northern Norfolk, and eastern Worcester counties; (3) *Quaboag Pond & River Complex*, comprising Brookfield, East Brookfield, and Spencer; (4) *Southern Connecticut River Valley*, comprising eight towns east of the River and south of the Holyoke Range; and (5) *Southwest*, consisting of southern Sheffield (Figure 1). The Southwest and Southern Connecticut River Valley regions appear to be extensions of continuous distributions into Connecticut, while the Northeast region continues northward into New Hampshire and Maine (Figure 2).

Unisexual *Ambystoma* co-occur with *A. laterale* throughout four of the five aforementioned Massachusetts regions (Tables 1, 2; Figure 3), except that the lineage is very rare (or quite possibly absent) from the Southeast (Bogart and Klemens 1997, 2008; Charney et al. 2014; Kubel 2016; NHESP unpublished data). In fact, unisexual *Ambystoma* are generally prevalent (and often dominant) in *A. laterale* populations throughout the northeastern United States (Figure 4), except in several small, geographically distinct regions consisting of southeastern Massachusetts, eastern Connecticut, eastern Long Island, and a site in new Jersey (Uzzell 1964;

Anderson and Giacosie 1967; Bogart and Klemens 1997, 2008; Charney et al. 2014; Kubel 2016; NHESP unpublished data). The extreme rarity (or possible absence) of the unisexual lineage in those regions makes their populations of *A. laterale* genetically unique, as there is little to no influence of *A. jeffersonianum* or *A. barbouri* genomes. Hence, for purposes of nomenclature in this Species Listing Proposal, local populations of *A. laterale* will be referenced by their apparent population structures:

(a) populations known or believed to contain significant proportions (e.g., >5%) of unisexual *Ambystoma* will be termed "*A. laterale* Complex populations", and

(b) populations known or believed to consist overwhelmingly or entirely (e.g., 95-100%) of pure *A. laterale* will be termed "*A. laterale*-dominated populations".

The example thresholds cited above are arbitrary and meant primarily to serve as a basis for nomenclature in the general categorization of population-level genetic structures. A hypothetical debate over where, exactly, a numerical threshold should be set to differentiate between *A. laterale*-dominated versus *A. laterale* Complex populations is essentially moot with respect to the Proposal, as the data presented herein show that there is no evidence that unisexual *Ambystoma* occur in the Southeast region of Massachusetts or, conversely, that they are anything but prevalent in all other regions of the state. That is, the data collected to date do not suggest that *A. laterale* population structures among any of the five regions even approach the hypothetical threshold of 5% unisexual composition. Population structure in the Southeast region is unique and very different from the other four regions.

Through isozyme electrophoresis, blood-cell analysis, and karyotyping, Bogart and Klemens (1997, 2008) were the first to suggest on the basis of broadscale sampling (i.e., across New England, New York, New Jersey, and Pennsylvania) that *A. laterale*-dominated populations occurred in southeastern Massachusetts. However, with only two of three known *A. laterale* populations sampled from that region of the state, and their sample sizes being relatively small (12 salamanders from one site, 6 from the other; Table 1), the idea warranted further investigation. Charney et al. (2014) analyzed DNA samples from 24 additional salamanders at one of those sites, and all (100%) were found to be pure *A. laterale* (Table 1). The NHESP extended the investigation in 2015, seeking out undiscovered populations in the region and analyzing DNA from a sample of 107 salamanders (range 1-79 per population) among four of the recently discovered sites (Kubel 2016, NHESP unpublished data). All (100%) of those salamanders were pure *A. laterale* (Table 1), supporting the belief that unisexual *Ambystoma* are extremely rare – if even present – in the Southeast region of Massachusetts. In contrast, unisexual *Ambystoma* are typically found at ≥60% rates at *A. laterale* sites in the other regions of the state (Table 2). At 86 *A. laterale* sites confirmed by Bogart and Klemens (1997, 2008) to host unisexual *Ambystoma* in the northeastern U.S., unisexuals were detected in the first 1 to 5 samples collected ≥83% of the time, demonstrating how little sampling is typically needed to determine presence of unisexuals within *A. laterale* Complex populations. Hence, failure to detect a single unisexual *Ambystoma* salamander among 149 DNA samples (and an additional 51 morphometric samples) in the Southeast region of Massachusetts (Table 1) is strong evidence that its populations of *A. laterale* are genetically distinct. The extreme paucity (or absence) of the unisexual lineage in southeastern Massachusetts is consistent with the pattern of *A. laterale* population structure observed in the broader region extending through southeastern New England and eastern Long Island (Table 1, Figure 4).

At present, there are 9 confirmed and 2 unconfirmed occurrences of *A. laterale* in the Southeast region of Massachusetts (Table 1, Figure 3). One confirmed occurrence is believed to be extirpated. Three confirmed occurrences occur very close together along the same river drainage and could arguably be considered a single local population. The two unconfirmed occurrences (circa 1980s) lack supporting documentation (e.g., photos and/or specimens), and NHESP research contractors and volunteers were unable to validate them during surveys conducted in 2016 and 2018. Thus, Massachusetts currently supports 6–8 confirmed *A. laterale*-dominated populations, representing approximately half of those documented in the northeastern U.S. (Table 1). I suspect at least several additional, undiscovered populations occur in Massachusetts, but survey work to date (Kubel 2016, NHESP unpublished data) suggests that the unique, *A. laterale*-dominated populations are rare and have a very restricted distribution. Based on the small number of occurrences and their extremely limited distributions throughout the broader region of the northeastern U.S. (Table 1, Figure

4), Massachusetts appears to have a very high regional responsibility for conservation of *A. laterale*-dominated populations.

(7) Trends.

Is the species decreasing (or increasing) in state distribution, number of occurrences, and/or population size? What is the reproductive status of populations? Is reproductive capacity naturally low? Has any long-term trend in these factors been documented?

The total number of documented occurrences of *A. laterale* has increased over the last several decades (NHESP Database 2018), but I believe that change is a cumulative result of continued reporting from the public and gradual increases in the scope and effectiveness of formal survey efforts, as opposed to increases in actual salamander abundance and/or distribution. Indeed, the number of newly-discovered occurrences each decade has been declining steadily since a peak during the 1980s (Figure 5), suggesting that fewer undocumented populations remain to be discovered. Given the increasingly high level of habitat fragmentation throughout most of the species' range in Massachusetts and the limited dispersal ability of *A. laterale* (see Section 8b below), there is little reason to suspect that populations will ever increase substantially in distribution without direct translocation efforts by wildlife managers.

Meanwhile, at least 6 documented local populations have very likely been extirpated as a result of habitat loss, isolation, and/or degradation, with another 30 not observed/reconfirmed in 25–40 years (NHESP Database 2018; Figure 1). Determination of trend in population size at the local level is generally cost-prohibitive, as sufficiently thorough and accurate sampling requires intensive (and expensive) efforts over many years, and the potential impacts of the sampling to salamanders (e.g., disrupted breeding) is not well understood. However, Windmiller et al. (2008) documented an apparent population decline of *A. laterale* complex salamanders during several years of study at a Sudbury site, with a loss of habitat to residential development the presumed cause. Anecdotally, numbers of *A. laterale* complex salamanders captured at a Wilbraham site declined substantially between two trapping efforts in the early 2000's and 2009 (Alan Richmond personal communication), but the methodology and effort involved do not allow for strong inferences there. I am not aware of any studies in Massachusetts that have demonstrated a population increase in *A. laterale*, nor any reason to suspect an upward population trend during the past several decades.

Of the 159 documented populations currently classified as extant, breeding activity has been confirmed at 88 (55%) (NHESP Database 2018). Given the generally strong natal site fidelity and limited dispersal ability in *Ambystoma* salamanders (Husting 1965, Whitford and Vinegar 1966, Semlitsch et al. 1993, Gamble et al. 2007, Homan et al. 2007), combined with high levels of habitat fragmentation at many *A. laterale* sites in Massachusetts, the great majority of documented occurrences in the state are presumed to signify presence of a breeding population. Reproductive capacity of pure *A. laterale* is not considered inherently low, but there has been much research and discussion among scientists regarding the influence of unisexual *Ambystoma* on reproductive capacity at the individual salamander and local population level. Embryonic mortality appears to be unusually high in egg masses of unisexual *Ambystoma* (Clanton 1934, Wilbur 1971, Licht 1989, JEK pers. obs.), and competition for sperm between pure *A. laterale* females and unisexuals could very well reduce productivity of the former (Minton 1954, Uzzell 1964, Lowcock et al. 1992). Unisexual *Ambystoma* almost invariably produce female offspring, and the offspring are most commonly clones of the unisexual mother (Spolsky et al. 1992, Bi et al. 2008, Ramsden 2008, Hoffman 2017). Therefore, how local populations of *A. laterale* complex salamanders are able to persist over time in the face of increasing proportions of unisexual salamanders has long been a mystery and topic of research (Clanton 1934, Minton 1954, Wilbur 1971, Bogart et al. 2017). Population crashes driven by shortages of males are conceivable (Clanton 1934, Minton 1954, Uzzell 1964) and, under normal circumstances, could be mitigated, "rescued", or re-colonized via immigration of pure individuals from nearby populations. However, anthropogenic habitat loss, construction of barriers to movement (e.g., highways), and the resulting isolation of populations over the past century has likely eliminated immigration into many local populations, possibly increasing the risk of unisexual *Ambystoma* becoming so dominant (and male *A. laterale* so scarce) at the local level that population crashes, or even extirpations, occur. Bogart et al. (2017) appear to be documenting such a phenomenon in an *A. jeffersonianum* complex population in southern Ontario, where the absence of suitable sperm donors is

causing gravid unisexuals to leave the breeding pond without depositing eggs, likely explaining a corresponding >50% decline in population size over a period of just 6 years. Hoffmann (2017) studied the demographics of four *A. laterale* complex populations in Maine, where unisexual *Ambystoma* were predominant and each population was seemingly supported by only 1 or 2 males (0.7–5.1% of total population size), thus raising questions about the viability of the populations. Other studies (Homan et al. 2007, Noël et al. 2011) have also inferred or hypothesized limited reproductive success in populations dominated by unisexual *Ambystoma*.

(8) <u>Threats and vulnerability.</u>

(a) What factors are driving a decreasing trend, or threatening reproductive status in the state? Please identify and describe any of the following threats, if present: habitat loss or degradation; predators, parasites, or competitors; species-targeted taking of individual organisms or disruption of breeding activity.

Although precise population trends have not been established, habitat loss to industrial, commercial, and residential development during the past century has almost certainly resulted in population loss and decreased salamander abundance at the local level. A review of historic records and aerial imagery confirms that at least 6 local populations have very likely been extirpated by development-related impacts (due to severe loss of habitat) and could very well explain why some of approximately 30 other populations have not been reconfirmed as extant within the past 25–40 years (NHESP Database 2018). In Priority Habitat alone, the NHESP has received approximately 20–80 project filings per year in habitat areas delineated for *A. laterale* since 2006 (totaling over 500 projects), thus demonstrating the persistent threat of habitat alteration and loss to the species in Massachusetts. Other primary threats to *A. laterale*, which have not been quantified in Massachusetts, include road mortality (Andrews et al. 2008, Fahrig and Rytwinski 2009); habitat degradation associated with roads (Turtle 2000, Karraker and Gibbs 2011, Brady 2012), urban development (Croteau et al. 2008, Snodgrass et al. 2008), and agricultural practices (Rohr et al. 2003, Freake and Lindquist 2008, Baker et al. 2013); and the spread of emerging infectious disease (Pico and Collins 2008, Gray et al. 2009). Population isolation resulting from habitat fragmentation has serious implications for population genetic structure and is likely to increase local extinction risks (Eastman et al. 2007, Greenwald et al. 2009). The potential influence of unisexuals on long-term reproductive success at the local level (see Section 7 above) is an additional threat in the Southwest, Southern Connecticut River Valley, Quaboag Pond and River Complex, and Northeast regions of Massachusetts. In the Southeast region, the threat of habitat loss is especially pronounced, as only 2 of the known *A. laterale* populations occur on predominantly protected land; habitat loss and fragmentation have likely extirpated 1 population and are substantial at 4 others.

(b) Does the species have highly specialized habitat, resource needs, or other ecological requirements? Is dispersal ability poor?

A. laterale has moderately specialized habitat and ecological requirements. The species requires relatively cool and moist microhabitats to avoid desiccation, which it accomplishes via a fossorial lifestyle in forested habitat (Petranka 1998). The species also requires fishless wetlands (with minimum hydroperiods of March/April through July/August in Massachusetts) to reproduce successfully. Therefore, habitat patches must have a combination of both upland forest and accessible, fishless wetlands. Spatially, aquatic breeding habitat typically occurs within several hundred meters of the occupied forest habitat (Regosin et al. 2005, Ryan and Calhoun 2014, Hoffmann 2017).

Being a flightless organism, *A. laterale* has very limited dispersal ability compared to a variety of other taxa (e.g., birds, bats, odonates, lepidopterans). *A. laterale* is short-legged, ectothermic, and vulnerable to desiccation, and so it is capable of only relatively short-distance movements during particular weather conditions (e.g., wet or humid). Long-range dispersal of Ambystomatid salamanders is further challenged by barriers such as highways (Andrews et al. 2008, Fahrig and Rytwinski 2009) and open spaces (deMaynadier and Hunter 1998, Regosin et al. 2005, Rittenhouse and Semlitsch 2006). Long-range dispersal of *A. laterale* may also be limited by natural habitat features associated with physiographic variables (e.g., surficial geology, elevation), as suggested by species occurrence data (NHESP Database 2018) and other accounts (Klemens 1993).

Conservation goals.

What specific conservation goals should be met in order to change the conservation status or to remove the species from the state list? Please address goals for any or all of the following:

(a) State distribution, number of occurrences (populations), population levels, and/or reproductive rates

No specific, formal goals have been developed.

(b) Amount of protected habitat and/or number of protected occurrences

No specific, formal goals have been developed.

(c) Management of protected habitat and/or occurrences

No specific, formal goals have been developed.

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Additional Comments/Summary

A. laterale-dominated populations in the Southeast region of Massachusetts are remarkably different from *A. laterale* Complex populations occurring elsewhere the state in terms of their genetic structure, and the *A. laterale*-dominated populations represent only 6% of the known, extant populations of *A. laterale* in Massachusetts. The *A. laterale*-dominated populations are likely isolated from the *A. laterale* Complex populations, based on a review of distribution data, geographic distances between regional populations, presence of artificial barriers to movement (e.g., highways) between those populations, and potential physiographic barriers (e.g., elevation, surficial geology) that may have been initial contributors to the apparent allopatry between population types. At a broader scale, *A. laterale*-dominated populations in the northeastern United States are known only from small geographic areas in southeastern New England, eastern Long Island, and a single site in northern New Jersey. Massachusetts populations represent over half of those known from the region. Therefore, the primary rationale for listing *A. laterale* as Threatened in Bristol and Plymouth counties is that (a) these unique populations are rare at the state level, (b) the populations are vulnerable to a suite of threats (primarily habitat loss and degradation), and (c) Massachusetts has very high responsibility for their regional conservation.

A. laterale Complex populations in the other regions of Massachusetts should remain listed as Special Concern on the basis of (a) their relatively rarity and (b) their vulnerability to a suite of threats. Of particular concern, but still poorly understood, is the risk of genetic bottlenecking and gradual loss of males over time in isolated populations where unisexual *Ambystoma* become increasingly dominant in the local population structure.

Connecticut already lists its *A. laterale*-dominated populations ("diploid populations") as Endangered, while its *A. laterale* Complex populations ("complex") are listed as Special Concern. Regionally within Connecticut, that equates to *A. laterale* populations in the eastern part of the state (Quinebaug watershed) being listed as Endangered and populations elsewhere (Connecticut River watershed, westward) listed as Special Concern.

Tables and Figures

See attached pages for Tables 1 and 2 and Figures 1 through 5.

Table 1. Documented samples (genetic, morphometric) from *Ambystoma laterale*-dominated populations identified in the northeastern United States. All (100%) of the known samples from the listed populations are "pure" *A. laterale* individuals (no unisexual *Ambystoma* were detected).

Population No.	State	Site[b]	DNA samples	Other Samples[d]	% Unisexual
1[a]	MA	Bogart & Klemens Site 61	12		0.0%
2	MA	Kubel Site ASD		2	0.0%
3	MA	Kubel Site ABR	79		0.0%
4	MA	NHESP EO 295			
5	MA	NHESP EO 127			
6	MA	NHESP Site ACB / EO 302	20		0.0%
7	MA	Kubel Site TTM	7		0.0%
8	MA	Charney Site 15; Bogart & Klemens Site 66	30	49	0.0%
9	MA	NHESP Site MCS / EO 303	1		0.0%
10	CT	CT-DEEP Site A			
11	CT	Bogart & Klemens Site 60	19		0.0%
12	CT	Bogart & Klemens Site 204[c]	20		0.0%
13	CT	Bogart & Klemens Site 57	11		0.0%
14	NY	Bogart & Klemens Site 71	44		0.0%
15	NJ	Bogart & Klemens Site 154	13	50[e]	0.0%
		MA Totals	**149**	**51**	**0.0%**
		Northeastern U.S. Totals	**256**	**101**	**0.0%**

[a] Population likely extirpated.
[b] Sites are from Bogart and Klemens (1997, 2008), Charney et al. (2014), Kubel (2016), Connecticut Department of Energy and Environmental Protection (pers. comm. 2018), and the NHESP Database (2018).
[c] Ryan and Calhoun (2014) cite an additional 164 unpublished samples of pure *A. laterale* in the vicinity by James Bogart.
[d] Additional individuals without DNA samples but for which body measurements (e.g., snout-vent length) or erythrocyte areas were recorded, following techniques and thresholds in Uzzell (1964) , Lowcock et al. 1992, and Charney et al. (2014).
[e] From Anderson and Giacosie (1967).

Table 2. Documented genetic samples from 33 *Ambystoma laterale* Complex populations among the Southwest (SW), Southern Connecticut River Valley (SCRV), Quaboag Pond & River Complex (QPRC), and Northeast (NE) regions of Massachusetts. Samples were analyzed to determine whether each individual was a "pure" *A. laterale* or a unisexual *Ambystoma*.

Population No.	Region	Site[a]	DNA samples	No. Pure *A. laterale*	No. Unisexual	% Unisexual
1	SW	Kubel Site SBC	14	1	13	92.9%
2	SW	Kubel Site SBA	61	29	32	52.5%
2	SW	Bogart & Klemens Site 216	1	0	1	100.0%
3	SW	Kubel Site SBN	80	31	49	61.3%
4	SCRV	Bogart & Klemens Site 59	1	0	1	100.0%
5	SCRV	Kubel Site SCA	5[c]	0	5[c]	100.0%
6	SCRV	Kubel Site SHP	5[c]	0	5[c]	100.0%
7	SCRV	Charney Site 8	7	0	7	100.0%
8	SCRV	Bogart & Klemens Site 64	8	0	8	100.0%
9	SCRV	Kubel Site SHB	5[c]	0	5[c]	100.0%
10	SCRV	Kubel/NHESP Site GBB	10	1	9	90.0%
11	QPRC	Bogart & Klemens Site 69	3	0	3	100.0%
12	QPRC	Bogart & Klemens Site 70	28	22	6	21.4%
13	NE	Charney Site 11	2	0	2	100.0%
14	NE	Charney Site 12	8	4	4	50.0%
15	NE	Bogart & Klemens Site 68	1	0	1	100.0%
16	NE	Charney Site 10	8	3	5	62.5%
17	NE	Bogart & Klemens Site 65	1	1	0	0.0%
18	NE	NHESP Site SPB / EO 129	10[c]	0	10[c]	100.0%
19	NE	Bogart & Klemens Site 67	13	2	11	84.6%
20	NE	Charney Site 9	4	0	4	100.0%
21	NE	Charney Site 13	5	2	3	60.0%
22	NE	NHESP Site GTF / EO 300	33	9	24	72.7%
23	NE	Bogart & Klemens Site 214	4	1	3	75.0%
24	NE	Bogart & Klemens Site 215	12	1	11	91.7%
25	NE	NHESP Site LLM / EO 96	5[c]	0	5[c]	100.0%
25	NE	NHESP Site LLM / EO 96	1	1	0	0.0%
26	NE	Bogart & Klemens Site 62	1	1	0	0.0%
27	NE	Bogart & Klemens Site 62	7	0	7	100.0%
28	NE	Bogart & Klemens Site 62	1	0	1	100.0%
29 & 30	NE	Bogart & Klemens Site 62	6	0	6	100.0%
30	NE	Bogart & Klemens Site 62	3	3	0	0.0%
31	NE	Bogart & Klemens Site 62	2	1	1	50.0%
32	NE	Bogart & Klemens Site 62	2	0	2	100.0%
33	NE	Charney Site 14	6	3	3	50.0%
		Total All Regions[b]	**338**	**116**	**222**	**65.7%**
		SW (n= 3 pops)	156	61	95	60.9%
		SCRV (n = 7 pops)	29	1	28	96.6%
		QPRC (n = 2 pops)	31	22	9	29.0%
		NE (n = 21 pops)	122	32	90	73.8%

[a] Sites are from Bogart and Klemens (1997, 2008), Charney et al. (2014), Kubel (2016), and the NHESP Database (2018).
[b] Totals treat all embryo samples from a site as a single sample, as independence among embryos could not be assured.
[c] Embryo samples; while each embryo was collected from a different egg mass, the egg masses are not necessarily independent (i.e., some could have been produced by the same female).

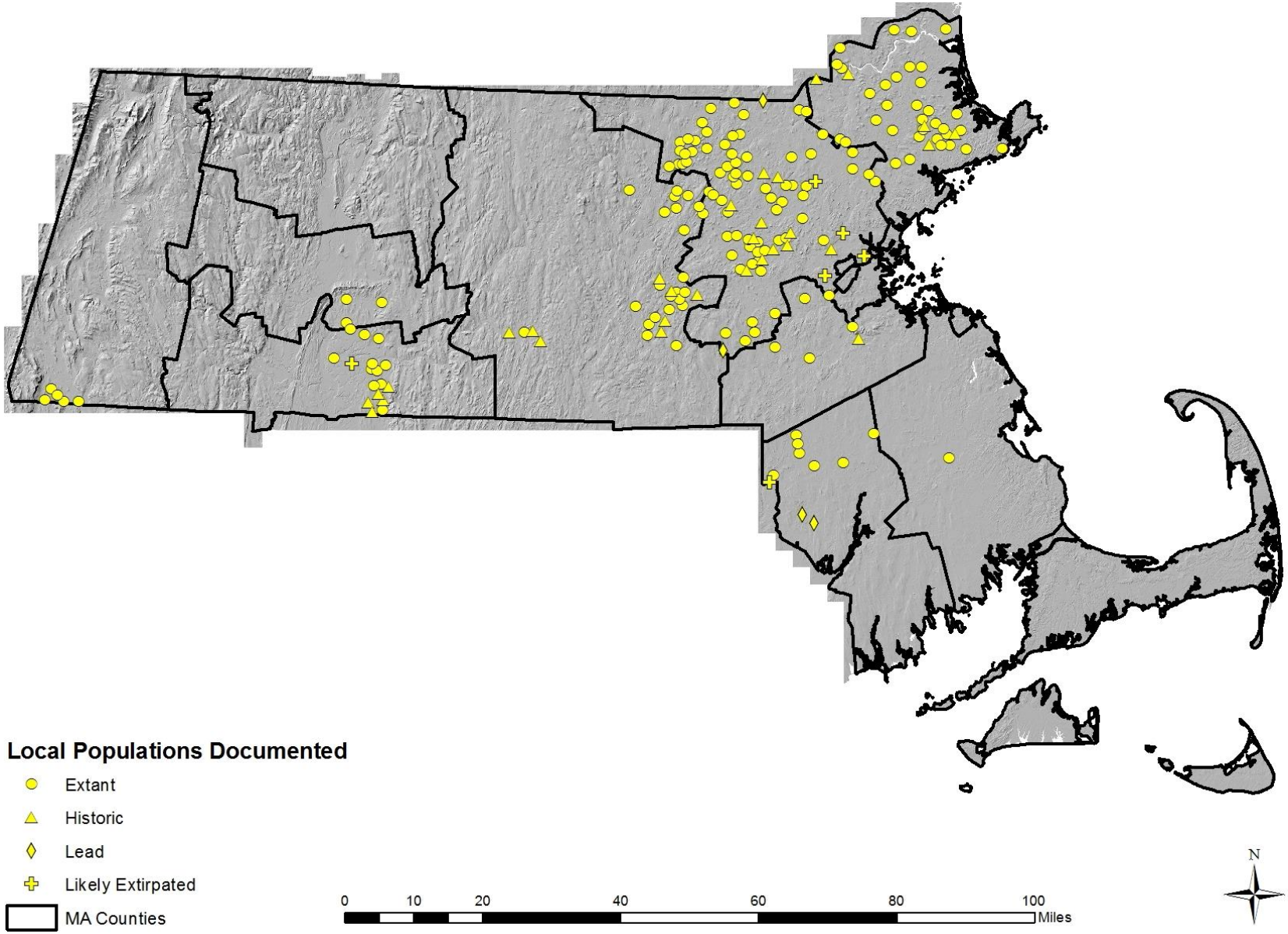

Figure 1. Distribution of Blue-spotted Salamander (*Ambystoma laterale*) populations in Massachusetts through 2017, as tracked in the NHESP Database (2018). Each population is classified as extant (observed within past 25 years; *n* = 159), historic (not observed within past 25–40 years; *n* = 30), lead (unconfirmed but credible report; *n* = 4), or likely extirpated (*n* = 6).

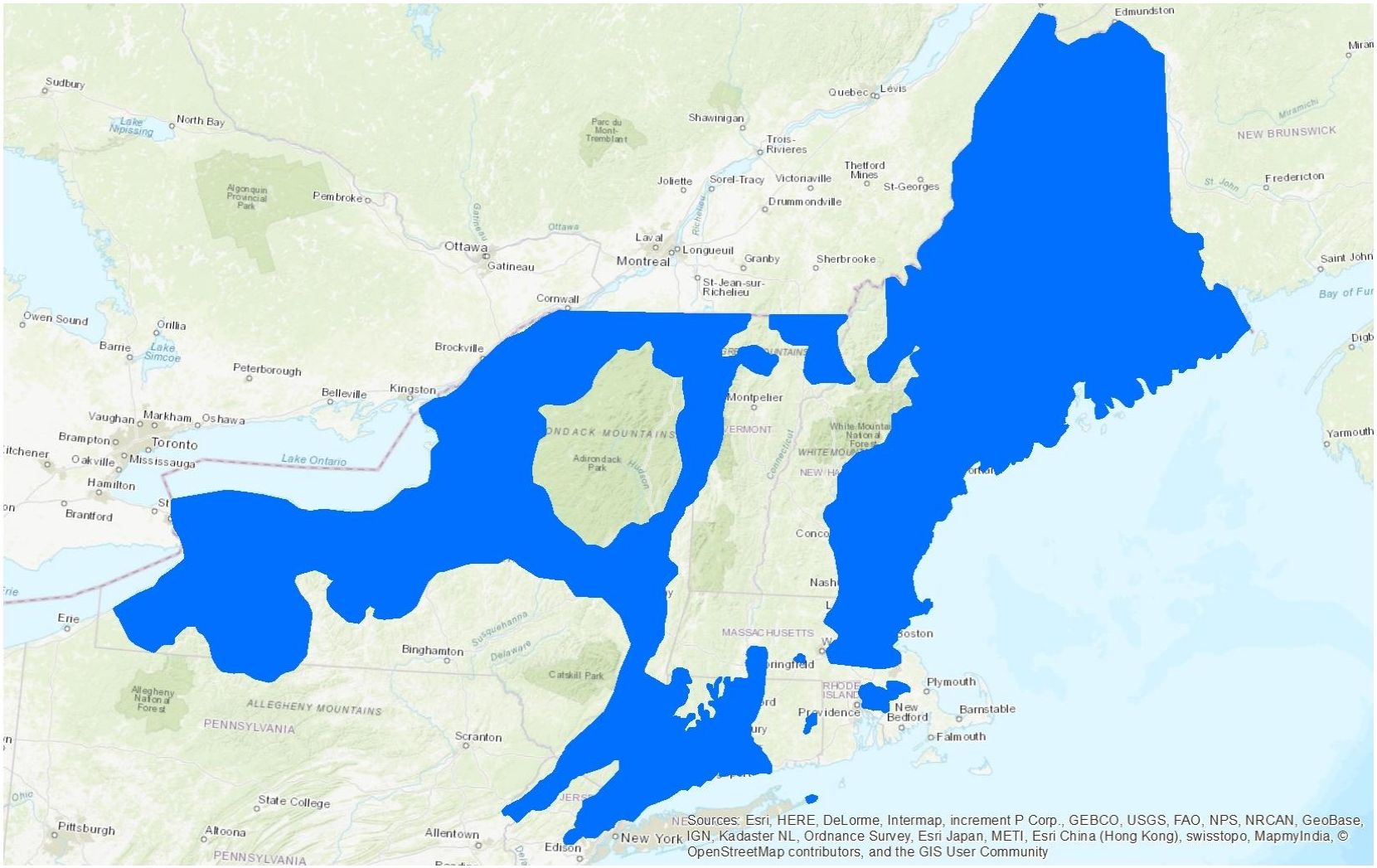

Figure 2. Geographic range of Blue-spotted Salamander (*Ambystoma laterale*) in the northeastern United States (range extends northward into Quebec and westward into Ontario). Adapted from Petranka (1998), Klemens (1993), Bogart and Klemens (1997, 2008), Bi et al. (2008), the NHESP Database (2018), and various state wildlife agency fact sheets, wildlife action plans, and herpetological atlases.

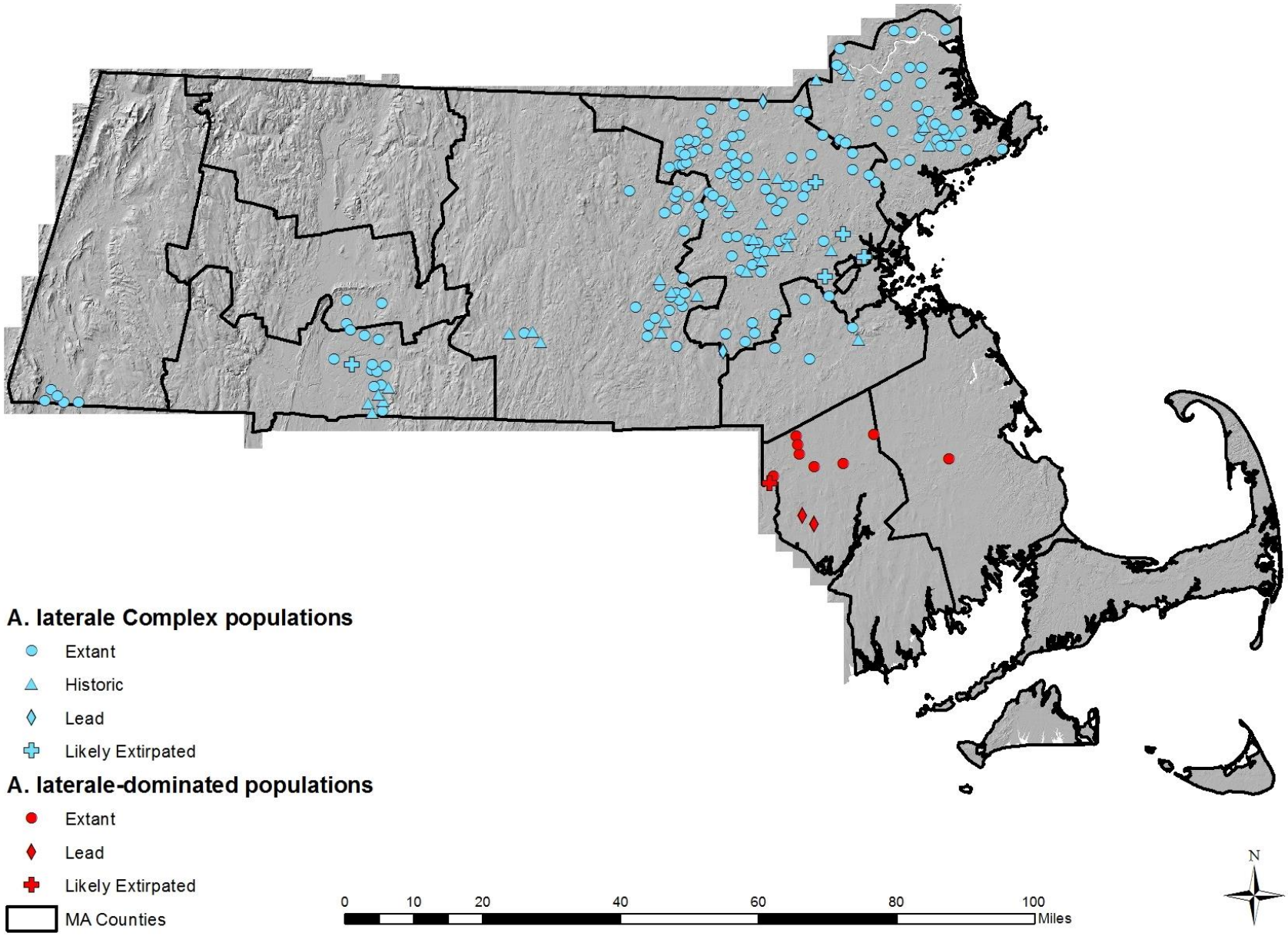

Figure 3. Distribution of *Ambystoma laterale* Complex populations and *A. laterale*-dominated populations in Massachusetts through 2017, as tracked in the NHESP Database (2018). Each population is classified as extant (observed within past 25 years), historic (not observed within past 25–40 years), lead (unconfirmed but credible report), or likely extirpated.

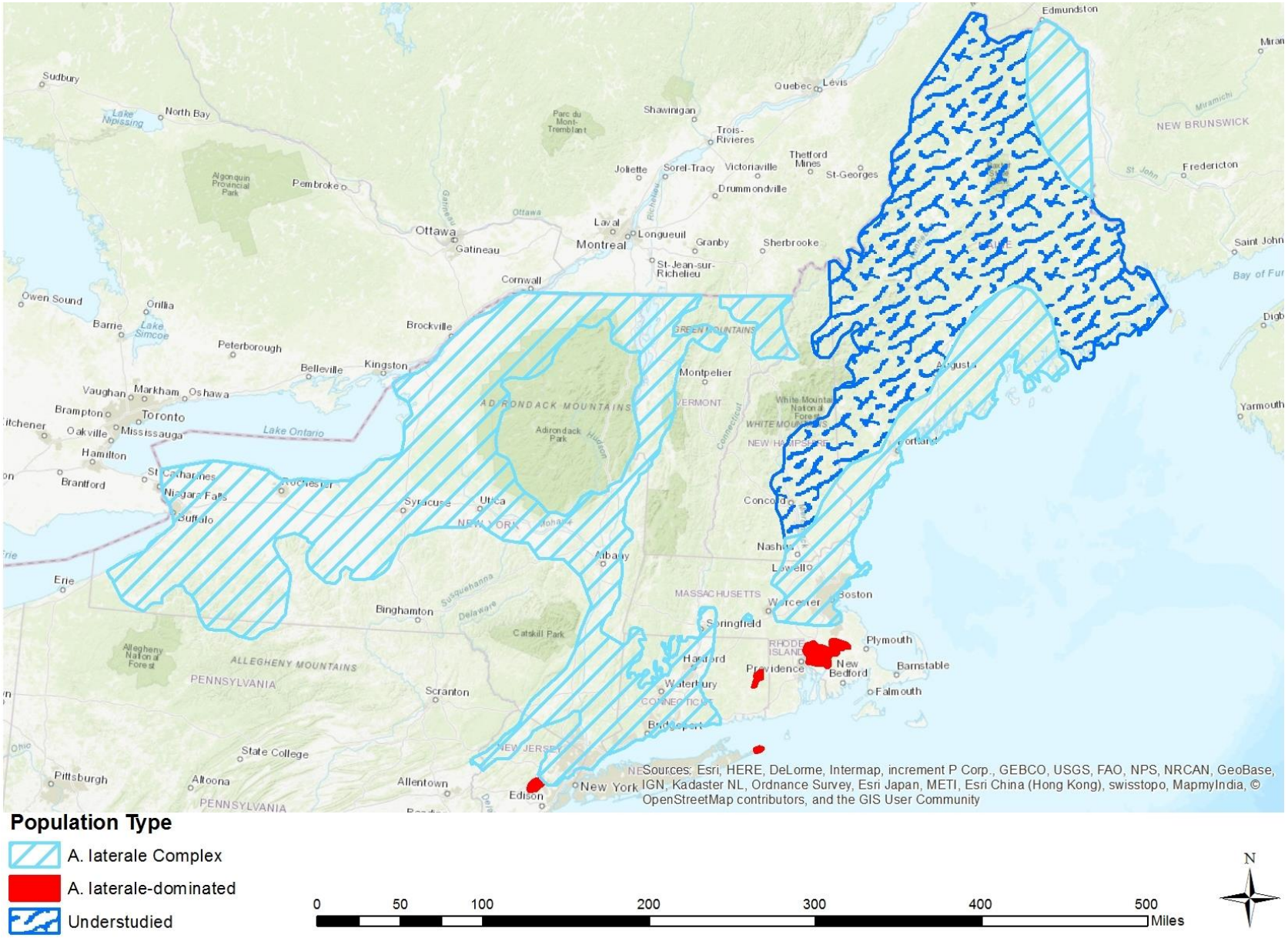

Figure 4. Distribution of *Ambystoma laterale* Complex populations and *A. laterale*-dominated populations in the northeastern United States. Adapted from Petranka (1998), Klemens (1993), Bogart and Klemens (1997, 2008), Bi et al. (2008), the NHESP Database (2018), and various state wildlife agency fact sheets, wildlife action plans, and herpetological atlases.

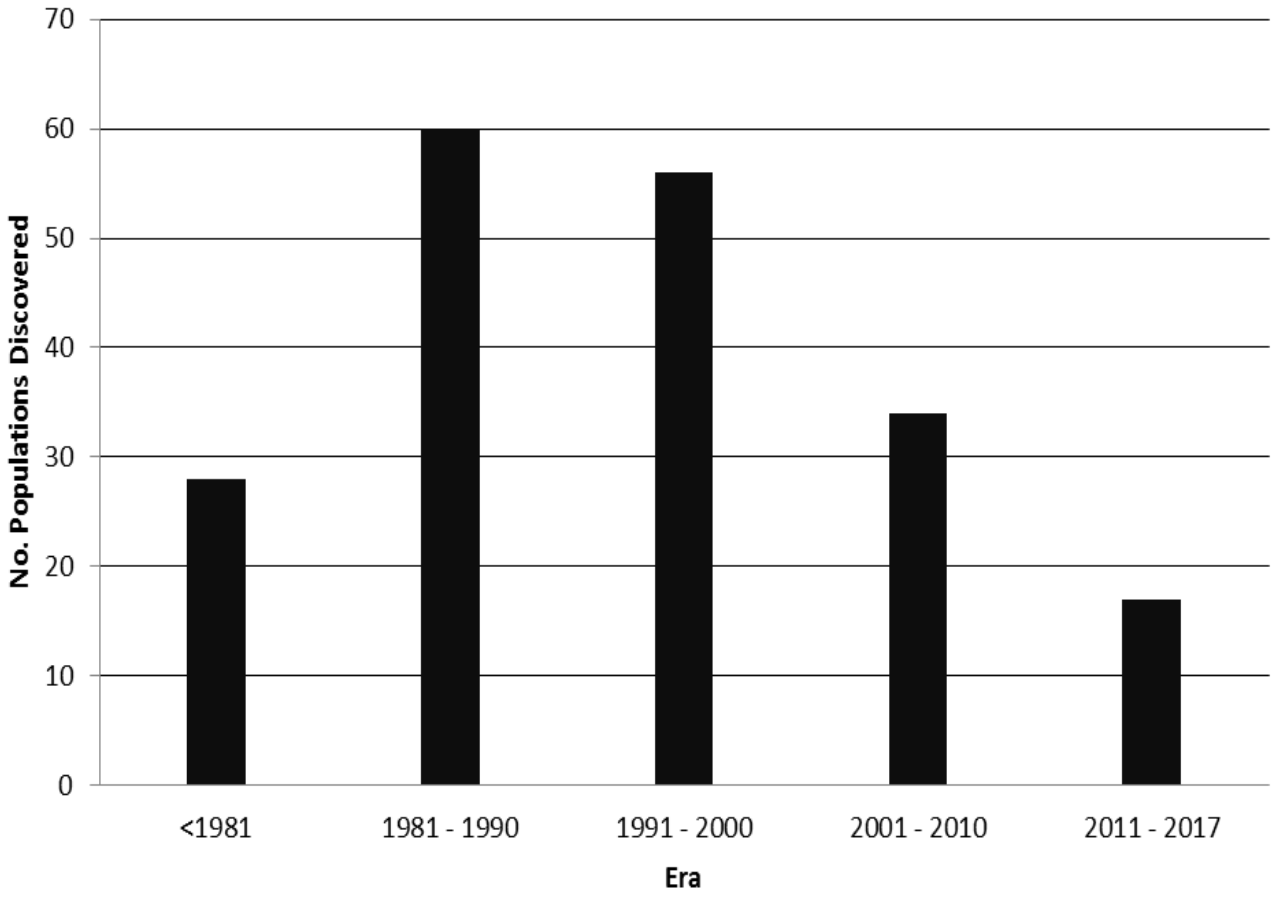

Figure 5. Number of newly discovered Blue-spotted Salamander (*Ambystoma laterale*) populations documented in Massachusetts, by era, based on occurrence records in the NHESP Database (2018). For each population (*n* = 195), the "discovery" date was assigned to the year in which the population was first observed, which was not necessarily the year it was first reported to the NHESP. Data set excludes leads.